

USE OF MOLT-ACCELERATING COMPOUNDS,  
ECDYSTEROIDS, ANALOGS THEREOF, AND  
CHITIN SYNTHESIS INHIBITORS  
FOR CONTROLLING TERMITES

Cross-Reference to Related Applications

This application claims the benefit of U.S. provisional application Serial No. 60/546,356, filed February 19, 2004.

Background of the Invention

Subterranean termites most often enter structures from the surrounding soil to feed on wood, or other cellulosic material, of the structure and its contents. If unchecked, termites can cause considerable damage. As a result, efforts to erect physical or chemical barriers to prevent the entrance of termites into a structure or to exterminate the termites after they have invaded a structure have proven a considerable expense to the public.

Subterranean termites construct an extensive foraging gallery beneath the soil surface. A single colony may contain several million termites with foraging territory extending hundreds of feet (Su, N.Y., R.H. Scheffrahn [1988] *Sociobiol.* 14(2):353-359). Because subterranean termites are cryptic creatures, their presence is not normally known until after some damage, foraging tubes, or live termites (such as swarmers) are found.

Currently, there are two basic approaches for the control of subterranean termites: preventive control and remedial control. In some of the United States, it is mandatory that the soil underlying the foundation of newly constructed buildings be pre-treated with a pesticide (also referred to herein as termiticide) to prevent termite infestation. However, the barrier often loses its continuity, and the currently available soil termiticides tend to lose their biological activity after a few years. Established termite colonies in the soil may then invade the structure if additional chemical is not applied beneath and around the structure.

When a house or other building is infested by subterranean termites, one option is to introduce termiticide around a building foundation by injection into soil underlying concrete foundations, drenching the soil surrounding the building perimeter, or a combination of both. This type of post-construction treatment is labor-intensive and may not adequately produce a continuous

barrier. Other remedial treatments include spot treatments such as dusting or injecting termicides (such as arsenic trioxide) within the walls of the building.

[0006] Some toxicants that have less environmental effect and that show activity against termites are known. However, prior to 1995, these toxicants were not used in conjunction with a method that efficiently and efficaciously delivered the toxicant to a target pest.

[0007] The introduction of the first commercial termite bait system, SENTRICON®, in 1995 drastically changed subterranean termite control practices. Unlike prior treatments, a monitoring-baiting program such as SENTRICON® is capable of eliminating the entire colony. See WO 93/23998, U.S. Patent No. 6,370,812, and U.S. Patent No. 6,397,516. Because of the target-specific approach, only a few grams of hexaflumuron are needed to eliminate a colony that may contain several hundred thousand termites (Su 1994, *J. Econ. Entomol.* 87:389-397). Due to its low environmental impact, hexaflumuron was the first compound to be registered under EPA's Reduced Risk Pesticide Initiative. The SENTRICON® system was the recipient of EPA's Presidential Green Chemistry Award in 2000.

[0008] There remains a need for additional termite toxicants that have little or no adverse environmental effects. For example, U.S. Patent Nos. 5,753,249; 6,214,364; and 5,558,862 relate to methods of controlling insects by administering enzymes that disrupt ecdysteroid metabolic pathways.

[0009] Insects have an exoskeleton (made of mostly chitin and proteins) that protects them from external elements such as weather and natural enemies. The external cuticle, however, has to be shed periodically for continuous growth. Endocrine products, most notably brain hormone, juvenile hormone, and ecdysone (**Figure 1A**), are known to regulate insect molting (Chapman 1976). Ecdysone is rapidly converted to 20-hydroxyecdysone (or "20E," **Figure 1B**) following its release into the insect hemolymph (Nation 2002). The presence of juvenile hormone (JH), a sesquiterpenoid, ensures that insects retain the juvenile form (*i.e.* to molt from a younger larval stage to the next larval stage). As insects progressively molt, JH concentration decreases and may even be totally absent at the last larval instar. In the absence of JH, a larva then molts into pupa or adult stage.

[0010] The effects of JH, its analogs (JHAs), and mimetics (JHMs) on termites are well studied (Su and Scheffrahn 1990). JHAs and JHMs (referred to as juvenoids) are known to produce excessive soldier termites whose function is for colony defense. Because the soldier caste has to

be fed by workers, termite colonies contain optimal proportions of the soldier caste (Wilson 1971, Haverty 1977). It has been proposed that juvenoids, which induce excessive soldier formation, may be used to disrupt the integrity of a termite society, leading to the destruction of the entire colony (Haverty 1977, Hrdy and Krecek 1972, Hrdy 1973). However, further studies revealed that juvenoids are effective only against termite species with a lower natural soldier proportion, such as *Reticulitermes* species (Su and Scheffrahn 1990). *Coptotermes* species, which have a relatively high proportion of soldiers, include an unusually large proportion of economically important termites in the world (Su 2003).

[0011] One relatively recent development in subterranean termite control is the use of termite baits containing chitin synthesis inhibitors (CSIs) such as hexaflumuron or noviflumuron to eliminate the vast colony of subterranean termites (Su 1994, Su 2003). In order to eliminate an entire colony of termites, the active ingredient (AI) for a bait has to be non-repellent, slow-acting, and its lethal time has to be dose-independent so that the AI is distributed throughout the colony by termites before the onset of death (Su and Scheffrahn 1998). Insect growth regulators (IGRs) such as juvenoids and CSIs satisfy all these three requirements. However, many IGRs are species-specific (Su 2003). CSIs generally inhibit the biosynthesis of chitin, but its complete process remains poorly understood (Nation 2002).

[0012] U.S. Patent No. 6,093,415 relates to synergistic effects between juvenoid insecticides and CSIs in termite baits. Ecdysone and analogs thereof are not mentioned.

[0013] While juvenoids and CSIs are well investigated for their potentials for termite control, limited information is available for even the normal function of ecdysone in termites. Lüscher and Karson (1958) and Lüscher (1960), while trying to determine the role, if any, that ecdysone plays in the normal biology of termites (and not in attempts to control termites), reported that injection of ecdysone alone or in combination with JH induced a normal pseudogate molting of the lower termites, *Kalotermes flavicollis*. Since their studies, there has been no known investigation of the effects of ecdysone in or on termites.

[0014] Synthetic versions of ecdysteroids have been used to control some insect pests of agricultural importance, but not termites. These analogs typically mimic the activity of 20E to cause premature molting (Wing *et al.* 1988). Toward the end of the molting under normal condition, 20E is degraded and excreted, thus allowing the eclosion hormone to complete the process (Nation 2002). The analogs, however, are more stable than 20E and are not easily

degraded or excreted (Wing *et al.* 1998). Consequently, their continuing presence in insect hemolymph interferes with complete molting and causes hyperecdysonism (premature molting without a successful termination). Hence, these analogs can be referred to as ecdysteroid agonists (Dhadialla *et al.* 1998).

[0015] U.S. Patent Nos. 6,123,756 and 6,248,159 relate to wood preservative for protecting wood against dry-wood-destroying insects, such as the house longhorn (*Hylotrupes bajulus*), woodworm (*Anobium punctatum*), and bark beetle (*Lyctus brunneus*). Those patents relate to lumber (not insect bait) treated with a combination of a juvenile hormone and an ecdysone agonist. Subterranean termites are different from drywood termites, are not dry-wood-destroying insects, and are not mentioned or suggested in those patents. See, e.g., U.S. Patent No. 5,027,546, which describes a system intended for use on above ground termites, i.e. drywood termites, by freezing them with liquid nitrogen.

[0016] Positive results were obtained when the ecdysteroid agonist RH-5849 was tested against some insect species (Darvas *et al.* 1992), but limited information is available regarding potential effects of ecdysteroid agonists against termites. Raina *et al.* (2003) reported that one such agonist, halofenozide, may impact the reproductive physiology of alate nymphs of *C. formosanus*. However, for use in baits to eliminate a subterranean termite colony, the active ingredient in the bait has to be lethal to the worker caste, which makes up the majority of the colony population. Alate nymphs, which make up only a small portion of a colony, eventually leave the nest to start a new colony somewhere else. However, they do not forage like workers. Thus, eliminating the young, alate nymphs would not impact the overall colony population and its damaging potential.

[0017] Halofenozide is one of several molt-accelerating compounds (MACs) owned by Dow AgroSciences. Halofenozide has activity on Lepidoptera and some Coleoptera species. In the urban pest management business, halofenozide is currently registered under the trade name MACH 2<sup>TM</sup> in the United States for control of white grub and surface feeding Lepidoptera in residential and commercial lawns and golf courses.

[0018] There have been some limited reports of testing of halofenozide against termites. For example, an abstract from the Florida Entomological Society 2002 Annual Meeting (Monteagudo & Su [2002]), related to preliminary results of a choice test conducted to examine preference, deterrence, and lethality of the insect growth regulator bait halofenozide on workers of the

eastern subterranean termites (*Reticulitermes flavipes*). The corresponding “ten-minute paper” stated that halofenozide was evaluated in a choice test for its potential as a bait toxicant against the eastern subterranean termite. Feeding blocks composed of wood slices were vacuum-impregnated with halofenozide at various concentrations, with feeding deterrence occurring at concentrations greater than 4,000 ppm. There was also a USDA inhouse memo that mentioned RH-0345 (a.k.a. halo $\ominus$ fenozide) as having a significant effect on ovarian development in alates and nymphs of *C. formosanus*. However, the effect, at low doses used in the test, was temporary. That memo also discussed the use of a juvenile hormone analog to induce the formation of mutant soldiers. The USDA termite program in New Orleans has also examined halofenozide for potential ovicidal effects in *C. formosanus* and presented results in 10-minute oral presentation at the 2001 meeting of the Entomological Society of America (paper 0269).

[0019] There have been no known reports regarding testing ecdysteroids or analogs thereof, other than halofenozide, for termite control. Furthermore, there are no known tests regarding the combination of the CSI + MAC (or ecdysteroids) classes of chemistry against insects, including termites.

#### Brief Summary of the Invention

[0020] The subject invention relates in part to controlling termites by the use of a chitin synthesis inhibitor (CSI), such as hexaflumuron and/or noviflumuron, and an ecdysteroid (and analogs thereof) or a molt-accelerating compound (MAC), such as halofenozide. The subject invention also relates to mixtures comprising these two active ingredients. The MAC / ecdysteroid analog induces a preliminary molting event in a termite worker, which then allows the CSI to disrupt the molt and cause mortality. The combination of these active ingredients, causing accelerated molting together with inhibition of chitin synthesis, is surprisingly shown herein to enhance activity against termites, as compared to either group of compounds alone.

[0021] The subject invention relates in part to the oral administration of ecdysteroids and analogs thereof to foraging subterranean worker termites. After eating ecdysteroids of the subject invention, termites were induced to molting. However, they could not complete the molting and eventually died. This is the first time that fatal effects of ecdisone against termites were demonstrated. Preferred ecdysteroids for use according to the subject invention are ecdisone, analogs thereof, and 20-hydroxyecdisone, for example. When used with a CSI according to the

subject invention, the combination surprisingly provides faster (and more complete) efficacy against termites than either insecticide group alone.

[0022] The subject invention includes all combinations of any given MAC insecticide (such as halofenozide and/or tebufenozide) together with any given CSI for control of all termite species. The subject invention also includes any of various types of formulations and delivery systems (e.g., baits, dusts, and liquid formulations).

#### Brief Description of the Figures

[0023] **Figure 1A** shows the chemical structure of ecdysone. **Figure 1B** shows its active hormone 20-hydroxyecdysone.

[0024] **Figure 2A** shows the chemical structure of tebufenozide, an ecdysone analog. **Figure 2B** shows the chemical structure of halofenozide.

[0025] **Figure 3** illustrates the effects of halofenozide and hexaflumuron on eastern subterranean termites.

[0026] **Figure 4** illustrates the effects of halofenozide and noviflumuron on eastern subterranean termites.

#### Detailed Description of the Invention

[0027] The subject invention relates in part to controlling termites by the use of a chitin synthesis inhibitor (CSI), such as hexaflumuron and/or noviflumuron, and an ecdysteroid (and analogs thereof) or a molt-accelerating compound (MAC), such as halofenozide. The subject invention also relates to mixtures comprising these two active ingredients. The MAC / ecdysteroid analog induces a preliminary molting event in a termite worker, which then allows the CSI to disrupt the molt and cause mortality. The combination of these active ingredients, causing accelerated molting together with inhibition of chitin synthesis, is surprisingly shown herein to enhance activity against termites, as compared to either group of compounds alone.

[0028] The subject invention includes all combinations of any given MAC insecticide (such as halofenozide and/or tebufenozide) together with any given CSI for control of all termite species. The subject invention also includes any of various types of formulations and delivery systems (e.g., baits, dusts, and liquid formulations).

[0029] The subject invention also relates in part to the oral administration of ecdysteroids and analogs thereof for controlling subterranean termites. When exposed to (after eating or otherwise ingesting) ecdysteroids of the subject invention, termites were induced to molt. However, they could not complete the molting and eventually died. This is the first known report of fatal effects of ecdysteroids against termites.

[0030] Compositions of the subject invention can also be used to cause other adverse (but desirable) effects on the target termites, such as cessation of feeding and overt toxicity in affected termites. In addition, an effective amount of the ecdysteroid can be used to induce molting; this is helpful when used to “synergize” or enhance the effects of a CSI, as discussed herein. The subject invention provides exciting new options for inhibiting termites (*i.e.*, killing them and preferably the entire colony, making them sick, preventing them from feeding on wooden structures, and the like).

[0031] Preferred ecdysteroids for use according to the subject invention are ecdisone, analogs thereof, and 20-hydroxyecdisone (“20E” or “20E-ecdyson”; the by-product of ecdisone usually created inside insects), for example. However, other ecdysteroids, in addition to ecdisone and 20E, can be fed (and/or administered in food, provided in baits, and the like) to termites according to the subject invention, as explained in more detail below. When used with a CSI according to the subject invention, the combination surprisingly provides faster (and more complete) efficacy against termites than either insecticide group alone.

[0032] It was quite surprising to find that ecdisone and other analogs thereof provided excellent termite control. This discovery was completely unexpected and surprising, especially noting prior studies where injection of ecdisone into termites simply caused successful molting without any harmful effects. (Lüscher and Karson 1958; Lüscher 1960.) Thus, in some embodiments of the subject invention, the ecdysteroid component can be used without the CSI component. Halofenozide, also called RH-0345, is a coleopteran-specific variant of tebufenozide. In embodiments where the ecdysteroid (or analog) is used without a CSI, halofenozide is excluded from such embodiments. With that noted, “molt accelerating compounds” as used herein include natural and synthetic compounds, ecdysteroids, analogs thereof, halofenozide, and all related insecticides that are useful and functional for inducing accelerated and/or premature molting in termites.

[0033] Summarizing some of the other results reported herein, a series of laboratory trials were conducted to determine the survivorship and feeding response of eastern subterranean termites (EST), *Reticulitermes flavipes* (Kollar) in oral ingestion choice tests with halofenozide. Tests were also conducted to evaluate the potential additive/synergistic effects of halofenozide (at various concentrations) when combined with the chitin synthesis inhibitors hexaflumuron or noviflumuron. The combination of halofenozide plus a CSI insecticide caused enhanced efficacy when compared to either type of insecticide alone. The impact of halofenozide appeared greater when combined with hexaflumuron, although a boost in speed of activity was also seen in combination with noviflumuron. For 5000-ppm hexaflumuron, a concentration of 5000-ppm halofenozide was needed to cause significant additive effects within 26 days. Lower concentrations of halofenozide (2500-1250 ppm) enhanced 5000-ppm noviflumuron within 14 days. A smaller-scale study on the termite *Amithermes wheeleri* also showed that halofenozide can enhance efficacy when combined with CSI insecticide.

[0034] Other examples of ecdysteroid analogs for use (or adaptable for use) according to the subject invention include tebufenozide, which is an insecticide originally designed for controlling lepidopteran pests. It does not affect natural populations of beneficial, predatory, and parasitic insects for the control of other insect pests. **Figure 2A** shows the chemical structure of tebufenozide, from the 1996 *Farm Chemicals Handbook*. RH-5849 (1,2-dibenzoyl-1-tert-butylhydrazine) is a beetle-specific alternative to tebufenozide. Halofenozide, also called RH-0345, is a coleopteran-specific variant of tebufenozide. Halofenozide is also a preferred MAC for use with a CSI according to the subject invention. **Figure 2B** shows the chemical structure of halofenozide. Some other ecdysteroid agonists include methoxyfenozide and chromafenozide. Thus, the subject invention can include the use of tebufenozide, tebufenozide analogues, RH-5849, and chromafenozide. Another possibility is methoxyfenozide or RH-2485.

[0035] It should be noted that while the term “MAC” is used throughout this specification, MACs can also be thought of as molt-inhibiting compounds. As described elsewhere herein, MACs can induce molting, but the molting is not completed. Thus, the term “molt-inhibiting” is also appropriate.

[0036] The “second” active ingredient (the “first” AI being a MAC, an ecdysteroid, or analog, of the subject invention) can preferably comprise chemicals that interfere with the formation of exocuticle such as the chitin synthesis inhibitors (CSIs). CSIs are known to interfere with the chitin

synthesis procedure, but such procedure does not take place until insects produce ecdysone under a pre-determined, natural biological clock. Thus CSIs have to passively wait for the natural molting to take place. When synergistically used in combination with CSIs, ecdysteroids or ecdysteroid agonists can induce termites to molt (after oral ingestion) even where the termites ingest a sublethal dose (a dose that may not cause hyperecdysionism but will initiate molting), and then the molting process is inhibited by a CSI. Examples of preferred second AIs, as mentioned above, are hexaflumuron, noviflumuron, diflubenzuron, flufenoxuron, chlorfluazuron, bistrifluron, azadirachtin, lufenuron, and other acryl ureas, which can be impregnated or incorporated into cellulose material, for example, during the formulation of the toxicant-containing matrix.

[0037] The subject ecdysteroid or analog “AI” (active ingredient) can be used in conjunction with other toxicants or AIs (a “third” AI, for example). The subject ecdysone-type AIs and other preferred AIs are slow-acting, lethal at concentrations which do not repel target insects, and capable of being combined with the matrix as described above.

[0038] It is intended that pests directly contacting or ingesting the subject toxicants will not be killed immediately but will travel to and/or through their colony to recruit other nestmates to the toxicant, thereby resulting in the control of large numbers of colony members. It is preferred that the pest die days, weeks, or even months after encountering the toxicant of the subject invention.

[0039] Compounds for use according to the subject invention can be used in the bait matrix of SENTRICON-like station housings, above-ground stations, and hermetically sealed baits. The subject compounds can be fed to foraging subterranean termite workers and delivered by them to nestmates of the same colony. (Unless specifically stated, when “termites” are referred to herein generically, such reference is to subterranean termites. Subterranean termites are different from drywood termites.)

[0040] Monitoring-baiting systems (like SENTRICON) and CSIs are relatively recent developments in subterranean termite control. Termite baits containing chitin synthesis inhibitors (CSIs), such as hexaflumuron or noviflumuron, can advantageously be used to eliminate the vast colony of subterranean termites (Su 1994, Su 2003), which is an accomplishment that could not be achieved by use of traditional liquid insecticides. The active ingredient (AI) for a bait has to be non-repellent, slow-acting, and its lethal time has to be dose-independent so that the AI is distributed throughout the colony by termites before the onset of death (Su and Scheffrahn 1998).

[0041] Although the slow-acting characteristic for CSIs against termites is desirable for the complete distribution of the AI into the vast colony for its elimination, the lengthy time required for the hexaflumuron baits to kill a colony could become its shortcoming on some occasions. The time period for such termite baits to eliminate a colony may be divided into three segments: 1) time required for the discovery of bait stations by termites, 2) time required for termites to ingest lethal dose, and 3) time required for termites to begin molting (thereby allowing the ingested CSIs to interfere with successful molting, leading to the death of the individual). The relatively more recent use of noviflumuron has shortened the 2<sup>nd</sup> segment (the time required to ingest a lethal dose). Noviflumuron is more lethal than hexaflumuron, and a smaller amount (and thus the time needed for ingesting lethal doses) is required to reach the lethal dose. Because the effects of CSIs do not take place until molting process begins, the 3<sup>rd</sup> time segment remains the same when CSIs are used.

[0042] In this invention, the hyperecdysonism of ecdysone and 20E against *R. flavipes* and *C. formosanus* is described for the first time. These two termite species represent the most economically important genera of termites in the world. According to the subject invention,ecdysteroids and ecdysteroid agonists can be used in bait matrices to cause delayed lethality of termite workers, leading to the elimination of a colony. Use of ecdysteroids or these ecdysteroid agonists has an advantage over CSIs because ecdysteroids actively induce molting instead of passively waiting for termites to molt before the effects can take place. Thus, the subject invention advantageously (and surprisingly) removes (or reduces) the 3<sup>rd</sup> segment of time required for termite colony elimination. Ecdysteroids or the subject ecdysteroid agonists can be also used synergistically in combination with CSIs in baits so that termites that are exposed to a sublethal dose are induced to molt, yet the molting process is inhibited by the CSI.

[0043] Certain ecdysone analogs, which are growth regulators, may be more effective against certain species of termites. Many insect growth regulators (IGRs) can also be species-specific. Thus, they can be selected and optimized for a given situation. Prior to the disclosure of the subject invention, ecdysteroids and analogs thereof, other than halofenozide, were not investigated for their ability to control termites. However, in light of the disclosure of the subject invention, one will now consider ecdysteroids, such as ecdysone, analogs thereof, and byproducts thereof for use in termite control programs. In contrast to the state of the art prior to the subject invention, the fact that the natural hormones ecdysone and 20E showed positive results will now

lead those skilled in the art to believe and expect that other ecdysone agonists, especially those having desirable structural characteristics, can now be used to advantageously control termites. In preferred embodiments, one can use ecdysone and 20E in baits to induce hyperecdysonism in foraging worker caste (and non-foraging by trophallaxis) subterranean termites.

[0044] With the foregoing considered, examples of termite species that can be targeted (selectively) by use of the subject methods include *Coptotermes formosanus*, *Reticulitermes flavipes*, *R. hesperus*, *R. virginicus*, *R. tibialis*, and *Heterotermes aureus*, as well as termite species of the families (and pest genera) Mastotermitidae (*Mastotermes* species), Hodotermitidae (*Anacanthotermes*, *Zootermopsis* species), Rhinotermitidae (*Coptotermes*, *Heterotermes*, *Reticulitermes*, *Psammotermes*, *Prorhinotermes*, *Schedorhinotermes* species), Kalotermitidae (*Glyptotermes*, *Neotermes*, *Cryptotermes*, *Incisitermes*, *Kalotermes*, *Marginitermes* species), Serritermitidae, and Termitidae (*Pericapritermes*, *Allodontermes*, *Microtermes*, *Odontotermes*, *Nasutitermes*, *Termes*, *Amitermes*, *Globitermes*, *Microcerotermes* species), Termopsidae (*Hodotermopsis*, *Zootermopsis* species), and other pest species of termites. Preferably, methods of the subject invention are used to target subterranean termites.

[0045] The subject invention can be practiced in many ways. Some preferred apparatuses are described in WO 93/23998, U.S. Patent No. 6,370,812, and U.S. Patent No. 6,397,516. Some embodiments of the subject invention can include a housing that is designed to enclose a monitoring device and/or toxicant-containing matrix. This housing is useful for protecting the monitoring device and/or toxicant-containing matrix from the environment. The monitoring device or matrix can be enclosed within the housing in such a manner so they can be removed with minimal disruption to the foraging termites. This housing is preferably made from a durable, non-biodegradable material. Preferably, once infested by termites, the monitoring device can be gently removed from the soil or from the station housing (it is advantageous to utilize a station housing to minimize disruption to foraging tunnels). Upon removal of the monitoring device, a toxicant-containing matrix, comprising an ecdysteroid or an analog thereof (and CSI if desired), can then be placed in the station housing. The monitoring device and the toxicant matrix preferably comprise cellulose.

[0046] Various materials can be used, if desired, to encase the toxicant-containing matrix. This method for packaging the toxicant-containing matrix can also be used to create "dose-packs" that precisely provide the appropriate amount of toxicant. "An effective amount" of the subject

ecdysteroid toxicant can be administered that is sufficient to kill, make sick, and/or prevent termite feeding of the structure or area being protected. “An effective amount” also distinguishes over naturally occurring (relatively very low) levels of the ecdysone or analog thereof that might be found in nature. An “effective amount” for termite control can also distinguish over natural or non-orally administered amounts of ecdysone that cause the normal termite molting process.

[0047] Depending on the MAC and CSI used (or if a CSI is used) and on the totality of circumstances for a given application, the ecdysteroid of the subject invention can be administered/made available to foraging worker termites (is present in the toxicant matrix) at a concentration of less than 10,000 ppm, at or below 7,500 ppm, at or below 5,000 ppm, and at or below 1,000 ppm. Also preferred are concentrations of less than 4,000 ppm. With these sample “ceilings” in mind, sample “floors” can be determined and include any of the concentrations specifically exemplified herein. For example, a preferred concentration range for embodiments using halofenozide (and depending on the CSI used) is 2500-5000 ppm.

[0048] Again, there are a variety of methods and apparatuses that can be utilized to practice the subject invention. The precise methods and apparatuses can be selected for optimal control of a particular target pest and environmental setting. Such applications would be apparent to a person skilled in this art using the teachings provided herein. For example, for particularly “shy” species of termites, a toxicant of the subject invention can be selected accordingly and used in the hermetically sealed baits described in WO 03/082000, US-2003-0177689-A1, and U.S. Patent No. 6,857,223. Bait stations using the subject toxicants can also be made more “attractive” to termites (generally and/or specifically) by using the pheromones and semiochemicals and non-cellulose polymer delivery devices of WO 03/092376 and US-2003-0180343-A1. Electronic monitoring can also be employed in some embodiments of the subject invention. See e.g. U.S. Patent Nos. 6,404,210; 6,052,066; and 5,815,090.

[0049] The subject invention includes combinations and mixtures of one or more types of MACs / ecdysteroids (and analogs) together with combinations and mixtures of one or more types of CSIs (both hexaflumuron and noviflumuron, for example). Furthermore, the two (or more) active ingredients (“AIs”) of the subject invention need not be co-administered or provided at exactly the same time. The subject invention includes sequential administration of at least one of the AIs followed by, in an effective time period to allow for the enhanced action of the subject invention, administration of at least one of the other class of AIs. For example, the MAC could be provided

first. At the desired time (for example, when sufficient time has elapsed to allow molting to be initiated), the CSI could then be provided. The CSI could also be provided first (to allow for dispersement in the colony, for example). The MAC/ecdysteroid/ecdysteroid analog could then be provided (for example, to initiate molting and to provide for enhanced kill / control of the termites). Factors such as timing and dosage can be optimized, according to the subject invention (for example, to effect control of the entire colony or colonies, to ensure adequate dispersal through the colony, and the like).

[0050] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[0051] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Protocol for feeding ecdysone to subterranean termites and determining the effects thereof

[0052] Termites were collected from three colonies each of *C. formosanus* and *R. flavipes* and were held in the laboratory at  $26 \pm 1^\circ\text{C}$  and 98 ± 2% RH before use. Technical grade ecdysone was dissolved in methanol to obtain 0.1, 1, 10, 100 and 1,000-ppm solutions by serial dilution. Methanol solutions (*i.e.*, 0 ppm AI) were used as untreated controls. Each concentration solution (0.2 ml) was pipetted onto 55-mm-diameter Whatman No. 1 filter paper fitted into 5-cm-diameter glass Petri dishes and allowed to evaporate overnight.

[0053] This filter paper served as the cellulose food source for twenty-five termites, plus one soldier for *R. flavipes* or three soldiers for *C. formosanus*, which were introduced into each Petri dish after moistening the filter paper with 0.175 ml deionized water. For each species, two subsamples each of three colonies were used per concentration for a total of 72 experimental units. The bioassay units was held at  $26 \pm 1^\circ\text{C}$ . Observation was made daily for 12 days. Termites showing symptoms of incomplete molting were counted, and dead termites were removed from each unit. Because affected termites did not recover, they were included in

mortality data. For each treatment and species combination, mean percent termites showing symptoms of incomplete molting and mortality among concentrations at 12 day were arcsine-root transformed and subjected to the analysis of variance (ANOVA). Significant differences ( $\alpha = 0.05$ ) among concentrations were separated using Fisher's least significant difference (LSD) test (SAS Institute 1999).

Example 2 – Results of feeding ecdysone to subterranean termites

[0054] Symptoms of hyperecdysonism (incomplete molting) were evident for both termite species after exposure to ecdysone at 1,000 ppm (**Table I**). After approximately 7 days of exposure, some termites exhibited the “jackknife” position due to the incomplete molting. The symptoms were similar to those exposed to the chitin synthesis inhibitor, hexaflumuron, as reported by Su & Scheffrahn (1993), or those observed from larval Coleopterans and Lepidoptera when treated with ecdysone agonists, where death occurs after premature molts are initiated but not terminated (Dhadialla *et al.* 1998). This is the only second time such incomplete molting symptom was recorded from termites (the first time being those caused by CSIs). With CSIs, termite molting was inhibited after ecdysone initiated the natural molting under a pre-set biological schedule. With the ecdysone exposure as described in this experiment, termites were artificially induced to premature molting without a successful termination. This is the reason why the symptoms of incomplete molting appeared faster in ecdysone (7 days) than in CSIs (4-8 weeks). Significant mortalities at 12 day were recorded for both termite species exposed to ecdysone at >100 ppm (Table I), with a large proportion of *R. flavipes* showing symptoms of hyperecdysonism. Surprisingly, 100% mortality was recorded from *C. formosanus* exposed to 100-ppm ecdyson. *C. formosanus* is generally less responsive to IGRs than *R. flavipes*, but with ecdyson, this appears to be the reverse.

**Table I.** Percent termites exhibiting incomplete molting ( $\pm$  SE) and mortality ( $\pm$  SE) of *R. flavipes* and *C. formosanus* after 12-day exposure to ecdysone.

Concentration (ppm)	% Incomplete Molting		% Mortality	
	<i>R. flavipes</i>	<i>C. formosanus</i>	<i>R. flavipes</i>	<i>C. formosanus</i>
0	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.64 $\pm$ 0.64a	0.60 $\pm$ 0.60a
0.1	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	1.79 $\pm$ 1.22a
1	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	1.19 $\pm$ 0.75a
10	0.00 $\pm$ 0.00a	2.38 $\pm$ 2.38a	0.00 $\pm$ 0.00a	11.31 $\pm$ 6.55a
100	0.00 $\pm$ 0.00a	8.33 $\pm$ 5.95a	60.26 $\pm$ 18.06b	100.00 $\pm$ 0.00b
1,000	21.80 $\pm$ 14.10b	3.57 $\pm$ 2.26a	100.00 $\pm$ 0.00c	93.45 $\pm$ 6.55b

Means within a column followed by the same letter are not significantly different ( $\alpha = 0.05$ ; ANOVA [SAS Institute 1999]).

Example 3 – Protocol for feeding 20-hydroxyecdysone to subterranean termites and determining the effects thereof

[0055] 20-hydroxyecdysone is an ecdysone byproduct. Technical grade 20-hydroxyecdysone (20E) was dissolved and used in the same manners as was ecdyonse, as discussed above in Example 1. The results are presented in the following example.

Example 4 – Results of feeding 20-hydroxyecdysone to subterranean termites

[0056] Symptoms of hyperecdysonism were evident for both termite species after exposure to 20E at 1,000 ppm (Table II). After approximately 7 days of exposure, some termites exhibited the “jackknife” position due to the incomplete molting, as discussed above in Example 2. As with ecdysone, 20E also caused incomplete molting at a faster rate than CSIs.

[0057] Significant mortality was recorded from *R. flavipes* exposed to >100 ppm 20E, with 100% mortality for those exposed to 1,000 ppm (Table II). *C. formosanus* was less responsive to 20E than *R. flavipes*, showing ca. 75% mortality at 1,000 ppm. Nonetheless, results of ecdysone and 20E were much superior than that for halofenozide, which caused only 50% mortality for *C. formosanus* at 10,000 ppm when tested under similar condition (Monteagudo and Su 2002).

**Table II.** Percent termites exhibiting incomplete molting ( $\pm$  SE) and mortality ( $\pm$  SE) of *R. flavipes* and *C. formosanus* after 12-day exposure to 20-hydroxyecdysone.

Concentration (ppm)	% Incomplete Molting		% Mortality	
	<i>R. flavipes</i>	<i>C. formosanus</i>	<i>R. flavipes</i>	<i>C. formosanus</i>
0	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	1.28 $\pm$ 0.81a	0.60 $\pm$ 0.60a
0.1	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.64 $\pm$ 0.64a	1.19 $\pm$ 0.75ab
1	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	10.12 $\pm$ 9.42bc
10	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.64 $\pm$ 0.64a	3.57 $\pm$ 2.26abc
100	3.85 $\pm$ 1.40ab	2.38 $\pm$ 1.19a	76.19 $\pm$ 11.28b	11.90 $\pm$ 5.43c
1,000	7.69 $\pm$ 3.29b	26.19 $\pm$ 6.09b	100.00 $\pm$ 0.00c	74.40 $\pm$ 3.62d

Means within a column followed by the same letter are not significantly different ( $\alpha = 0.05$ ; ANOVA [SAS Institute 1999]).

Example 5 - Additive Effects of the MAC Insecticide Halofenozide when Combined with Chitin Synthesis Inhibitors (Hexaflumuron and Noviflumuron) and Tested in Feeding Choice Tests with Eastern Subterranean Termites (EST)

This Example summarize a series of laboratory trials that determined the survivorship, and feeding response of eastern subterranean termites (EST), *Reticulitermes flavipes* (Kollar) in oral ingestion choice tests with halofenozide. Tests were also conducted to evaluate the potential additive/synergistic effects of halofenozide (at various concentrations) when combined with the chitin synthesis inhibitors hexaflumuron or noviflumuron.

**Test #1. Choice Feeding Bait Test, Halofenozide + Hexaflumuron.** A paired choice feeding test was conducted to compare feeding response and resultant mortality caused by various combinations of technical grade halofenozide and hexaflumuron treated on filter paper (acetone carrier). The test apparatus consisted of two round plastic containers connected by a 7-cm long piece of 2.5-mm diameter (ID) tygon tubing. A known number of termite workers were introduced into a central harborage chamber (5.5 cm round container with ventilated lid) containing a vermiculite/sand/water mixture of ca. 1:1:1. The central harborage chamber was connected by the tygon tubing to the bait foraging chamber (without sand/vermiculite matrix) containing the paired feeding choices.

ESTs were given the choice to feed between untreated blocks of southern yellow pine (SYP) and treated filter paper. Halofenozide was tested individually at concentrations of 1250, 2500, and 5000 ppm. Hexaflumuron was tested at 5000 ppm, and also combined with each of the aforementioned concentrations of halofenozide. For comparative purposes, technical grade noviflumuron were also tested at 5000 ppm. After twelve days, all treatments were removed,

oven-dried, and weighed for consumption. Filter paper was placed in all bioassay units that were held for fourteen more days. Surviving termites were then counted in each replicate. Thus, survival was counted at 26 days after initial exposure to the toxicant. Each treatment was replicated four times with 50 termites per replicate.

[0059] EST showed either significantly positive or neutral feeding response to all treatments on filter paper (FP) (**Table III**). As single treatments, 5000-ppm noviflumuron caused significantly greater efficacy than 5000-ppm halofenozide or 5000-ppm hexaflumuron after 26 days, while 5000-ppm halofenozide was significantly more efficacious than 5000 ppm hexaflumuron. The combination of 5000 ppm halofenozide + 5000 ppm hexaflumuron caused a significantly lower level of survivorship when compared to either treatment alone. Moreover, the combination of halofenozide + hexaflumuron was significantly equal to the level of efficacy provided by 5000 ppm noviflumuron. Note that combining 5000 ppm hexaflumuron with lower concentrations of halofenozide did not cause significantly enhanced efficacy over 5000 ppm hexaflumuron alone. See **Figure 3**, which graphically illustrates data from Table III.

<b>Choice<sup>a</sup></b>	<b>mg consumed after 12 days (Mean ± SEM)<sup>b</sup></b>	<b>No. Survivors/50 after 26 days (Mean ± SEM)<sup>c</sup></b>	<b>% Surviving</b>
Acetone Control FP vs. SYP	24.67 ± 4.52 a 8.98 ± 3.25 b	41.00 ± 4.49 ab	82.0
Noviflumuron FP (5000) vs. SYP	16.17 ± 3.64 a 1.77 ± 1.71 b	5.00 ± 3.72 e	10.0
Hexaflumuron FP (5000) vs. SYP	21.20 ± 2.06 a 9.28 ± 3.55 b	32.5 ± 5.48 bc	65.0
Halofenozide FP (5000) vs. SYP	9.33 ± 1.41 a 14.97 ± 3.85 a	23.25 ± 7.88 d	46.5
Halofenozide FP (2500) vs. SYP	18.70 ± 4.32 a 7.92 ± 2.13 a	33.75 ± 6.56 bc	67.5
Halofenozide FP (1250) vs. SYP	16.05 ± 3.39 a 9.49 ± 2.57 a	45.00 ± 2.04 a	90.0
Hex (5000) + Halo FP (5000) vs. SYP	13.92 ± 1.53 a 6.45 ± 2.20 b	11.25 ± 3.75 e	22.5
Hex (5000) + Halo FP (2500) vs. SYP	13.06 ± 2.58 a 13.27 ± 2.43 b	26.25 ± 2.56 cd	52.5
Hex (5000) + Halo FP (1250) vs. SYP	21.53 ± 5.32 a 5.97 ± 3.44 a	27.25 ± 6.65 cd	54.5

<sup>a</sup>Each choice test replicated four times (50 termites/rep)

<sup>b</sup>Within each choice test, pairs followed by the same letter are not significantly different (LSD; p > 0.10)

<sup>c</sup>Means followed by the same letter are not significantly different (LSD; p > 0.10)

[0060] **Test #2. Choice Feeding Bait Test, Halofenozide + Noviflumuron.** A standard paired choice feeding test using the same methods described in Test #1 was conducted to compare feeding response and resultant mortality caused by various combinations of technical grade halofenozide and noviflumuron treated on filter paper (acetone carrier). EST were given the choice to feed between untreated blocks of southern yellow pine (SYP) and treated filter paper. Halofenozide and noviflumuron were tested individually at concentrations of 5000 ppm. Five thousand ppm (5000-ppm) noviflumuron was also tested in combination with 1250, 2500, or 5000 ppm halofenozide. After fourteen days, all treatments were removed, oven-dried, and weighed for consumption. Because of the rapid activity of noviflumuron, surviving termites were also counted in each replicate after only 14 days of feeding. Each treatment was replicated four times with 50 termites per replicate.

[0061] Similar to results in Test #1, there was no significant repellency noted for any treatment, although there was numerically less acceptance of FP with 5000-ppm halofenozide (**Table IV**). After only fourteen days, 5000 ppm noviflumuron had already provided highly significant efficacy (only 27% survivorship). The single treatment of 5000-ppm halofenozide was also causing significant impact on survivorship after 27 days. However, the addition of halofenozide to noviflumuron appeared to boost speed of activity even further, especially when the two lowest concentrations of halofenozide were added. This can also be seen graphically in **Figure 4**. The most effective combination was 5000 ppm noviflumuron + 2500 ppm halofenozide, which caused significantly greater efficacy than either 5000 ppm of noviflumuron or halofenozide tested alone.

**Table IV.** Noviflumuron + Halofenozide Test: Comparative Consumption and Resultant Survivorship of EST in Choice Tests Between Untreated SYP and Treated FP.

<b>Choice<sup>a</sup></b>	<b>mg consumed after 14 days (Mean ± SEM)<sup>b</sup></b>	<b>No. Survivors/50 after 14 days (Mean ± SEM)<sup>c</sup></b>	<b>% Surviving</b>
Acetone Control FP vs. SYP	25.88 ± 3.70 a 5.15 ± 1.00 b	43.75 ± 3.61 a	87.5
Noviflumuron FP (5000) vs. SYP	14.58 ± 2.20 a 2.58 ± 1.03 b	13.50 ± 3.52 bc	27.0
Halofenozide FP (5000) vs. SYP	10.12 ± 1.48 a 8.35 ± 3.91 a	22.25 ± 5.36 b	44.5
Novi (5000) + Halo FP (5000) vs. SYP	7.53 ± 1.14 a 8.56 ± 4.52 a	8.50 ± 5.44 cd	17.0
Novi (5000) + Halo FP (2500) vs. SYP	8.28 ± 2.45 a 4.94 ± 2.24 a	3.00 ± 3.00 d	6.0
Novi (5000) + Halo FP (1250) vs. SYP	15.14 ± 2.20 a 1.63 ± 2.50 b	4.75 ± 0.95 cd	9.5

<sup>a</sup>Each choice test replicated four times (50 termites/rep)

<sup>b</sup>Within each choice test, pairs followed by the same letter are not significantly different (LSD; p > 0.10)

<sup>c</sup>Means followed by the same letter are not significantly different (LSD; p > 0.10)

[0062] **Summary.** Results of these studies indicate that the combination of halofenozide plus a CSI insecticide can cause enhanced activity when compared to either type of insecticide alone. The impact of halofenozide appeared greater when combined with hexaflumuron, although a boost in speed of activity was also seen in combination with noviflumuron. For 5000-ppm hexaflumuron, a concentration of 5000-ppm halofenozide was needed to cause significant additive effects within 26 days. Lower concentrations of halofenozide (2500-1250 ppm) enhanced 5000-ppm noviflumuron within 14 days. While halofenozide might have a tendency to cause less acceptance of termite bait when compared to hexaflumuron or noviflumuron alone, significant repellency was not observed for any concentration of halofenozide in this study.

Example 6 - Additive Effect of the MAC Insecticide Halofenozide when Combined with Noviflumuron and Tested in Forced Feeding Tests with *AmiTermes wheeleri*.

[0063] This Example summarize a laboratory trial that determined the survivorship, and feeding response of *AmiTermes wheeleri* in oral ingestion choice tests with halofenozide, noviflumuron and combination of the two active ingredients. *AmiTermes wheeleri* is in the termite family

(Termitidae), which is a different classification than the eastern subterranean termite (family Rhinotermitidae) shown in Example 5.

[0064] A forced feeding test was conducted to compare mortality caused by different active ingredients treated on purified cellulosic bait. The test apparatus was the same as that described in Example 5. Noviflumuron was tested on the bait at 5000-ppm concentration, and also in combination with 5000-ppm Halofenozide. Blank bait was also tested. Surviving termites were also counted at 28 days after initial exposure to the toxicant. Noviflumuron reduced termite survivorship in half when compared to the blank control. However, the combination of noviflumuron and halofenozide caused 100% kill, which again suggests that halofenozide can enhance the speed of efficacy for CSI insecticides.

**Table V.** Hexaflumuron + Halofenozide Test: Comparative Consumption and Resultant Survivorship of *Amithermes wheeleri* in Forced Feeding Bait Tests.

Treatment	No. Survivors/30 after 28 days	% Surviving
Blank Cellulosic Bait	26	87
Cellulosic Bait with 5000 ppm noviflumuron	12	43
Cellulosic Bait with 5000 ppm noviflumuron + 5000 ppm halofenozide	0	0

References

- Chapman, R. F. 1976.** The insects. Structure and function. Elsevier, New York.
- Darvas, B., L. Polgár, M. H. Tag El-Din, K. Eröss & K. D. Wing. 1992.** Developmental disturbances in different insect orders caused by an ecdysteroid agonist, RH 5849. J. Econ. Entomol. 85: 2107-2112.
- Dhadialla, T. S., G. R. Carlson & D. P. Le. 1998.** New insecticides with ecdysteroidal and juvenile hormone activity. Annu. Rev. Entomol. 43: 545-569.
- Haverty, M. I. 1977.** The proportion of soldiers in termite colonies: a list and a bibliography (Isoptera). Sociobiology 2: 199-216
- Hrdý, I. 1973.** Effect of juvenoids on termites and honeybees. Proc. 7th Int'l. Congr. IUSSI, London 1973, pp.158-161.
- Hrdý, I. & J. Křeček. 1972.** Development of superfluous soldiers induced by juvenile hormone analogues in the termite, *Reticulitermes lucifugus santonensis*. Insectes Soc. 19: 105-109.
- Lüscher, M. 1960.** Hormonal control of caste differentiation in termites. Ann. New York Acad. Sci. 89: 549-563.
- Lüscher, M. & P. Karlson. 1958.** Experimentelle auslösung von häutungen bei der termite *Kalotermes flavicollis* (Fabr.). J. Insect Physiol. 1: 341-345.
- Monteagudo, E., and N.-Y. Su. 2002.** Evaluation of halofenozide as a bait toxicant against eastern subterranean termite (Isoptera: Rhinotermitidae). Annual Mtng. Entomol. Soc. America, Ft. Lauderdale, FL. Nov. 17 -20.
- Nation, J. L. 2002.** Insect Physiology and Biochemistry. CRC Press, Boca Raton, FL.
- Raina, A. K., Y. I. Park & Z. Hruska. 2003.** Ecdysone agonist halofenozide affects corpora allata and reproductive physiology of the Formosan subterranean termite, *Coptotermes formosanus*. J. Insect Physiol. 49: 677-683.

**SAS Institute.** 1999. SAS/STAT guide for personal computers, 8<sup>th</sup> ed. SAS Institute, Cary, NC.

**Su, N.-Y.** 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 87: 389-397.

**Su, N.-Y.** 2003. Baits as a tool for population control of the Formosan subterranean termite. *Sociobiology* 41: 177-192.

**Su, N.-Y., & R. H. Scheffrahn.** 1990. Potential of insect growth regulators as termiticides: A review. *Sociobiology* 17: 313-328.

**Su, N.-Y., & R. H. Scheffrahn.** 1993. Laboratory evaluation of two chitin synthesis inhibitors, hexaflumuron and diflubenzuron, as bait toxicants against Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* 86: 1453-1457.

**Su, N.-Y., & R. H. Scheffrahn.** 1998. A review of subterranean termite control practices and prospects for integrated pest management programs. *Integrated Pest Management Reviews* 3: 1-13.

**Wilson, E. O.** 1971. *The Insect Societies*. Belknap Press, Cambridge, MA.

**Wing, K. D., R. A. Slawecki & G. R. Carlson.** 1988. RH 5849, a nonsteroidal ecdysone agonist: Effects on larval Lepidoptera. *Science* 241: 470-472.